The American Journal of
PATHOLOGY

JULY 1969 • Volume 56, Number 1

Nonspecific Green Birefringence in Congo Red-Stained Tissues

Gerald Klatskin, MD

AMYLOID DEPOSITS stained with Congo red characteristically exhibit green birefringence when examined microscopically between crossed polarizers.[1-4] The rotation of polarized light and interference with the transmission of red light involved in this phenomenon appear to depend on the fibrillar structure of amyloid and the orderly parallel arrangement that Congo red molecules assume when they are bound lengthwise to the fibrils of amyloid.[5,6] Most authorities consider green birefringence a pathognomonic sign of amyloidosis and recommend polarization microscopy of Congo red-stained tissues as the most specific and sensitive method for detecting amyloid deposits.[1-4] Although several investigators have suggested that fluorescence microscopy of thioflavin-T-stained tissues may be more sensitive,[7,8] only one report has questioned the specificity of the Congo red polarization method.[8]

The present study was prompted by the unexpected finding of green birefringence in a Congo red-stained needle biopsy section of normal human liver fixed in Carnoy's solution. Further investigation revealed that, under appropriate conditions of fixation, staining, illumination, and orientation of the sections, foci of green birefringence could be demonstrated in the connective tissues and/or blood vessels of virtually all normal and diseased livers and of many other normal organs both in man and in the rat.

From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Supported by Research Grant AM 05986 from the Institute of Arthritis and Metabolic Diseases, NIH, US Public Health Service.

Accepted for publication April 10, 1969.

Address for reprint requests: Dr. Gerald Klatskin, Yale University School of Medicine, 333 Cedar St, New Haven, Conn 06510.

Material and Methods

Tissues

After fixation by a variety of methods to be described, the following tissues were embedded in paraffin and sectioned at 5-μ intervals.

Amyloid Controls. Four needle biopsy specimens of liver from 3 patients with primary and 1 patient with secondary amyloidosis; and postmortem sections of liver, spleen, and kidney from 11 patients with amyloidosis (primary 2, secondary 9).

Nonamyloid Liver Tissue. A total of 72 needle biopsy specimens (normal 10, Laennec's cirrhosis 10, postnecrotic cirrhosis 10, biliary cirrhosis 6, pericholangitis and cholestasis secondary to biliary obstruction 10, viral hepatitis 11, and viral subacute hepatic necrosis 15); 15 surgical biopsy specimens (Laennec's cirrhosis 2, postnecrotic cirrhosis 1, biliary cirrhosis 3, and pericholangitis and cholestasis secondary to biliary obstruction 9); 8 postmortem specimens (biliary cirrhosis 3, pericholangitis and cholestasis secondary to biliary obstruction 1, and viral massive hepatic necrosis 4).

"Normal" Tissues Obtained at Necropsy. Multiple tissues from 4 infants and 1 young adult with no evidence of systemic amyloidosis.

Normal Rat Tissues. Multiple tissues from a 23-day-old, 60-g male weanling and a 400-g young male adult.

Fixation

The various fixatives employed are indicated in the text, and include Carnoy's solution,[9] Enzyk's Zenker-acetic,[9] Helly's Zenker-formol,[9] 10% formalin, Bouin's solution,[9] and absolute ethyl alcohol.

Staining

Consecutive sections of each block of tissue were stained by the following methods: Harris's hematoxylin and eosin,[9] Masson's trichrome,[9] Pearse's modified Congo red,[10] Puchtler's alkaline Congo red,[6] and Vassar's thioflavin-T.[11]

Microscopy

The instrument employed in all studies was a Zeiss binocular Photomicroscope fitted with planapochromat objectives, Complan eyepieces, a 1.3 Z condensor fitted with removable neutral density filters, a 12-v 60-w projection illuminator, and a centered rotating stage.

For polarization microscopy, a rotating polarizer screen (Zeiss 47-36-00) was placed on the substage illuminator, and a nonrotating analyzer (Zeiss 47-36-61) inserted just above the objectives. To ensure the detection of small deposits of green birefringence, polarization microscopy was carried out under conditions of maximal light intensity with the condensor focused to provide Koehler illumination. The iris diaphragm was then constricted until the intensity and brilliance of green birefringence were at peak levels. As will be discussed further, the intensity of illumination proved critical in the detection of small amounts of green birefringent material. By actual measurement, using a Gossen Microsix-L meter fitted with a cadmium sulfide detector probe, the light intensity at the level of the oculars in the Zeiss Photomicroscope was four times as great as that in a Leitz Ortholux binocular microscope fitted with a 6-v projection illuminator bulb. All sections showing green birefringence were photographed on 35-mm Kodachrome IIA film, using the automatic camera provided in the instrument.

For fluorescence microscopy of thioflavin-T-stained sections, the microscope was fitted with a Zeiss fluorescence illuminator (HBO 200-w high-pressure mercury bulb), a BG 12 exciter filter, and a 47 barrier filter.

Results

Amyloid Controls

On polarization microscopy, all Congo red-stained sections of tissue containing amyloid deposits exhibited foci of green birefringence (Table 1). These were best visualized at relatively low magnification (40–100×) and under conditions of intense illumination with constriction of the iris diaphragm of the condensor to achieve maximal contrast. Under such conditions, amyloid deposits appeared as compact arrays of sharply defined, brilliantly illuminated, deep blue-green fibers of varying length and breadth, located within connective tissue structures or the walls of blood vessels (Fig 1). At higher magnification (250–400×), or with the iris diaphragm fully open, the birefringent fibers appeared pale green or yellow-green in color. Reducing the intensity of illumination by introducing a series of graded neutral density filters at the light source resulted in progressive loss of green birefringence, an effect noted first in small groups of fibers visualized under high magnification. Storage of Congo red-stained sections of amyloid-containing tissue for periods of 12–18 months had a similar, although less striking, effect.

In any given orientation of the section within the beam of polarized light, only a fraction of the amyloid present exhibited green birefringence. However, on rotating the section through an arc of 90° in the horizontal plane, new foci of birefringence appeared as others were quenched (Fig 2). Indeed, unless such rotation was carried out routinely, small deposits of amyloid escaped detection. Another characteristic feature of the birefringence was its sudden change in color from green to red when the polarizers were uncrossed by a few degrees (Fig 3).

As shown in Table 1, green birefringence was demonstrable in Congo red-stained tissues preserved in any of the four fixatives employed. Unfortunately, the same fixative was used for all tissues in any given case, so that the influence of fixation on birefringence could not be evaluated.

Neither the intensity nor the distribution of the green birefringence differed significantly in adjacent sections stained with Puchtler's and Pearse's Congo red, respectively (Table 1). Similar, but usually less extensive, birefringence was demonstrable in some sections stained with hematoxylin and eosin, but never in Masson trichrome-stained sections.

All amyloid deposits that exhibited green birefringence also fluoresced

Table 1. Green Birefringence* and Thioflavin-T Fluorescence* in Tissues Containing Amyloid Deposits

Subject†	Liver				Kidney				Spleen			
	Green birefringence				Green birefringence				Green birefringence			
	Congo red	Alk. Cr	H&E	Thio-T fluor	Congo red	Alk. Cr	H&E	Thio-T fluor	Congo red	Alk. Cr	H&E	Thio-T fluor
Carnoy's Solution Fixation (Needle Biopsy Sections)												
1P	1+		1+	2+								
2P	2+		0	4+								
3P	1+		±	1+								
4S	2+		2+	2+								
Zenker-Formol Fixation (Necropsy Sections)												
5P	1+	1+	0	3+	2+	2+	1+	3+	3+	3+	2+	4+
6P	2+	3+	±	4+	1+	2+	±	3+	3+	3+	1+	4+
7S	3+	3+	0	3+‡	3+	2+	1+	3+‡	3+	2+	1+	3+‡
8S	2+	2+	1+	3+	3+	3+	2+	3+	±	0	0	±
9S	3+	3+	1+	3+‡	3+	3+	2+	3+‡	2+	3+	2+	2+
Zenker-Acetic Fixation (Necropsy Sections)												
10S					2+	3+	2+	4+	3+	3+	2+	4+‡
11S	3+	1+	0	±	3+	1+	0	1+				
12S					3+	2+	1+	2+	3+	3+	2+	4+‡
13S	±	1+	±	2+	2+	2+	0	3+	1+	3+	±	4+
Formalin Fixation (Necropsy Sections)												
14S	3+	3+	±	3+	3+	2+	±	2+	3+	2+	±	3+
15S	±	±	0	2+	2+	3+	1+	4+				

* Graded 0 to 4+ on basis of extent of amyloid deposition: ± indicates minute deposits, 4+ indicates massive deposits.
‡ Fluorescence faint, but extensive in distribution.
† P, primary amyloidosis; S, secondary amyloidosis.

when stained with thioflavin-T (Table 1). Neither the distribution nor the extent of the deposits differed significantly in adjacent sections examined by polarization and fluorescence microscopy, respectively.

Nonamyloid Liver Tissue

As shown in Table 2, green birefringence was demonstrable in 71 of 72 randomly selected needle biopsy sections of liver fixed in Carnoy's solution and stained with Congo red. None of the patients involved, who ranged in age from 18 to 83 years, had evidence of amyloidosis. Included in the group of sections studied were those of both normal liver and livers with a variety of lesions including Laennec's cirrhosis, postnecrotic cirrhosis, biliary cirrhosis, pericholangitis and cholestasis secondary to biliary obstruction, acute viral hepatitis, and viral subacute hepatic necrosis.

In all cases, the foci of green birefringence were found within connective tissue elements or vessel walls, and, with few exceptions, at multiple sites, including portal triads 82%, the walls of central veins 53%, sinusoids 47%, portal veins 11%, and cirrhotic septums 43% (Fig 4–6). Similar, but usually smaller, foci were also demonstrable in 10 of 71 adjacent sections stained with hematoxylin and eosin.

In all respects, the green birefringence was indistinguishable from that observed in amyloid deposits. However, in contrast to the latter, none of the areas of green birefringence found in these liver biopsy sections exhibited fluorescence in adjacent sections stained with thioflavin-T.

As in the case of amyloid deposits, prolonged storage of Congo red-stained sections led to diminution or loss of green birefringence.

A total of 15 surgical liver biopsy specimens of liver fixed in formalin and 8 necropsy specimens of liver fixed in Helly's Zenker-formol were

Table 2. Distribution of Nonspecific Green Birefringence in Congo Red-Stained Sections of Needle Biopsy Specimens of Liver Fixed in Carnoy's Solution

Diagnosis	No.	Green birefringence					
		Present	Central vein	Portal vein	Triad ct	Reticulin	Septal ct
Normal	10	10	9	0	10	7	—
Laennec's cirrhosis	10	10	1	3	9	6	9
Postnecrotic cirrhosis	10	10	1	4	7	6	8
Biliary cirrhosis	6	6	0	0	5	0	4
Biliary obstruction	10	9	3	0	9	3	1
Acute viral hepatitis	11	11	11	0	8	2	—
Viral subacute hepatic necrosis	15	15	13	1	11	10	9
Total	72	71	38	8	59	34	31

available for comparison with previously obtained needle biopsy specimens of liver fixed in Carnoy's solution. Whereas green birefringence was demonstrable in all 23 Carnoy's-fixed Congo red-stained needle biopsy sections, it was present in only 9 of the 15 formalin-fixed and 1 of the 8 Zenker-formol-fixed sections.

In contrast to amyloid-containing tissues, none of the hematoxylin and eosin-stained sections of liver exhibited green birefringence.

"Normal" Tissues Obtained at Necropsy

To establish whether or not green birefringence could be demonstrated in the connective tissues and blood vessels of organs other than the liver, multiple tissues obtained at necropsy were examined. To avoid microdeposits of amyloid attributable to senescence and certain disease states, which are said to be found in a high proportion of routine necropsies,[12,13] the cases selected for study included 4 infants and 1 young adult with no evidence of amyloidosis or degenerative disease: Patient 1 (B.J.), a 715-g female infant, who was born prematurely and died within 1 hr; except for collapsed lungs and other signs of prematurity, necropsy revealed no abnormalities; Patient 2 (H.G.), a term male infant, who died on the ninth day of life of renal failure attributable to polycystic kidneys; no other abnormalities were found post mortem; Patient 3 (A.T.), a term male infant, who died on the ninth day of life of heart failure attributable to congenital heart disease; necropsy revealed dextrocardia, truncus arteriosus, pulmonary venous obstruction, and signs of passive congestion, but no other abnormalities; Patient 4 (K.M.), an apparently healthy female infant, who died unexpectedly at the age of 5 weeks; the only abnormalities found post mortem were enlargement of the thymus and atrophy of the adrenals; Patient 5 (K.R.), a healthy 20-year-old woman, who died within a few hours of suffering a gunshot wound through the chest; in addition to the penetrating bullet wound and signs of hemorrhage, necropsy revealed a normal 5-month pregnancy.

In all 5 patients, scattered foci of green birefringent material, varying both in size and distribution, were demonstrable in the connective tissues and/or blood vessels of a high proportion of Congo red-stained sections obtained from numerous sites (Table 3). With one exception, all such foci were found in tissues fixed in Carnoy's, Zenker-acetic, or Zenker-formol solution (Fig 7–9). Of 20 sections fixed in formalin, only one exhibited minimal green birefringence.

As in the case of Carnoy's-fixed needle biopsy specimens of liver, none of the birefringent foci exhibited fluorescence in adjacent sections stained with thioflavin-T. However, fluorescence, typical of that

Table 3. Nonspecific Green Birefringence in Congo Red-Stained Tissues of Premature and Newborn Infants and a Young Healthy Pregnant Woman

Tissue*	Patient 1	Patient 2				Patient 3	Patient 4	Patient 5	
	(Z–F)	C	Z–A	Z–F	F	(Z–F)	(Z–F)	Z–F	F
Liver	1+	2+	2+	2+	0		3+	1+	
Spleen	0	1+	1+	1+	0		2+	0	
Kidney	1+						1+	1+	0
Adrenal	2+	±	3+	1+	0		2+		
Pancreas		1+	2+				3+	1+	
Esophagus		2+	3+	3+	0			2+	
Stomach							3+		
Jejunum	0	3+	3+	3+					
Heart	0	1+	2+	3+	0	2+	2+	±	0
Aorta		2+	3+	3+	0				
Lung	1+	±	2+	3+	0		3+	±	0
Thyroid							3+	3+	0
Thymus	0	0	2+	2+	0	2+	1+		
Lymph node						2+			
Skin	3+	3+	3+		0	3+	3+	1+	1+
Skeletal muscle	1+	±	2+	1+	0		2+	±	
Nerve		1+	1+		0	3+	3+		
Cartilage	3+					2+			
Bone	2+					2+			
Ovary								0	0
Vagina									0
Uterus									0
Breast								2+	
Placenta	2+								0
Umbilical cord	3+								
Sclera	3+							2+	0

* Green birefringence was demonstrated in connective tissue elements of these sections, and only rarely in blood vessel walls.
Fixatives: Z–F, Zenker-formol; C, Carnoy's solution; Z–A, Zenker-acetic; F, formalin.
See text for description of patients.

seen in amyloid deposits, was observed in other structures, including the elastic membranes of arteries and arterioles, surface keratin of skin, the centers of some pancreatic acini, and the Hassall bodies of the thymus.

Normal Rat Tissues

Adjacent, relatively thin slices of various tissues from a healthy 60-g weanling and a 400-g young adult rat were fixed in a variety of solutions and studied by polarization microscopy to confirm the occurrence of green birefringence in normal tissues, and to determine whether or not such birefringence is affected by the type of fixation employed.

As shown in Table 4, scattered foci of typical green birefringence of variable size and distribution were found in the connective tissues and/or blood vessels of almost all Congo red-stained sections fixed in Carnoy's, Zenker-acetic, Zenker-formol, absolute ethyl alcohol, or Bouin's

solution (Fig 10–12). In contrast, only one section fixed in formalin exhibited such birefringence. A number of sections exhibited atypical birefringence that was either bright, light yellow-green or dull grey-green in color.

Green birefringence was not observed in any of the hematoxylin and eosin-stained sections fixed in formalin. However, minute foci were encountered rarely in similarly stained sections preserved in other fixatives (Table 4).

Irrespective of the type of fixation or staining, none of the foci of green birefringence fluoresced in adjacent sections stained with thioflavin-T. However, fluorescence typical of that seen in amyloid deposits was observed at a number of other sites, including the elastic membranes of some arterioles, droplets within the epithelial cells of renal tubules and casts within their lumens, the centers of pancreatic acini, some skeletal muscle fibers, hair follicles, the mucosal surface of the jejunum and the trachea, and cartilage of the ear.

Discussion

The evidence presented indicates that, under appropriate conditions of fixation, illumination, and orientation, Congo red-stained sections of

Table 4. Nonspecific Green Birefringence in Rat Tissues

	400-g male rat										60-g male rat	
	Congo red stain					H&E stain						
Tissue*	C	Z–A	B	A	F	C	Z–A	B	A	F	Cr stain and C fixative	H&E stain and C fixative
Liver	1+	1+	1+	3+	0	0	0	0	0	0	2+	0
Spleen	3+	3+	3+	3+	0	0	0	0	0	0	3+	0
Kidney	1+	1+	1+	2+	0	0	0	0	0	0	3+	0
Adrenal	2+	3+	2+	1+	0	0	0	0	0	0	1+	0
Pancreas	3+	?	±	1+	0	0	0	0	0	0	—	—
Esophagus	?	?	3+	3+	±	?	?	0	0	0	3+	0
Jejunum	3+	2+	3+	3+	0	0	0	0	0	0	2+	0
Heart	3+	?	1+	±	0	±	0	0	0	0	2+	0
Aorta	?	?	3+	3+	0	?	?	0	0	0	—	—
Trachea	?	2+	?	?	?	?	0	?	0	?	—	—
Lung	±	±	2+	1+	0	0	0	0	0	0	3+	0
Skin	3+	2+	3+	?	?	0	0	0	?	0	1+	0
Adipose tissue	3+	2+	?	1+	0	0	0	?	0	0	?	?
Skeletal muscle	3+	2+	3+	3+	?	0	0	0	±	0	—	—
Tendon	?	?	3+	?	?	0	0	0	0	0	1+	0
Nerve	—	2+	—	—	0	0	0	0	0	0	—	—
Testis	±	1+	0	0	0	±	±	0	0	0	1+	0

* Green birefringence was demonstrated in connective tissue elements of these sections and only rarely in blood vessel walls. Graded 0 to 4+ on basis of distribution of green birefringence; ? indicates atypical birefringence—either light yellow-green or dull grey-green.

Fixatives: C, Carnoy's solution; Z–A, Zenker-acetic; B, Bouin's solution; A, absolute alcohol; F, formalin.

many tissues exhibit foci of green birefringence in the absence of amyloidosis. It is evident, therefore, that green birefringence cannot be considered a pathognomonic sign of amyloidosis.

In contrast to amyloid deposits, foci of nonspecific green birefringence do not fluoresce when stained with thioflavin-T. Moreover, their occurrence in the healthy tissues of newborns and young adults, both in man and the rat, rules out the possibility that they are related to the focal microdeposits of amyloid found in senescence and certain disease states.[12,13]

Foci of nonspecific green birefringence were demonstrable in a wide variety of tissues fixed in Carnoy's, Zenker-acetic, absolute alcohol, or Bouin's solution, but were found only rarely in similar tissues fixed in formalin or Zenker-formol. In contrast, amyloid deposits invariably exhibited green birefringence irrespective of the method of fixation. However, adjacent blocks of tissue preserved in different fixatives were not available for comparison, so that the possibility cannot be excluded that formalin fixation partially inhibits green birefringence in amyloid deposits. Puchtler, Sweat, and Levine [6] have reported that Congo red stains amyloid more intensely when tissues are fixed in Carnoy's solution or absolute alcohol than when they are fixed in formalin or Zenker-formol, and these authors have suggested that formaldehyde, by reacting with the hydroxyl groups of amyloid, interferes with dye binding. Since the phenomenon of green birefringence appears to be dependent on the spacing and orderly parallel arrangement that Congo red molecules assume when they are bound lengthwise to arrays of amyloid fibrils,[5,6] it might be anticipated that formaldehyde, by interfering with dye binding, would reduce the green birefringence of amyloid, and might inhibit it completely in small deposits.

The fact that formalin inhibited nonspecific green birefringence strongly suggests that such birefringence, as in the case of amyloid deposits, is attributable to binding of Congo red by parallel arrays of appropriately spaced fibrils. Although all foci of nonspecific green birefringence were found in connective tissue structures or blood vessel walls, their distribution was far more limited than that of collagen, reticulin, or elastic fibers. It is highly improbable, therefore, that dye binding by such fibers accounts for nonspecific green birefringence. A more likely, although still speculative, possibility is that nonspecific green birefringence is attributable to binding of Congo red by the parallel arrays of extracellular microfibrils that are normally found intermixed with collagen and elastic fibers, or closely associated with fibroblasts and basement membranes.[14] Janigan [15] has pointed out the

ultrastructural similarities between such microfibrils and the fibrils of amyloid and has speculated that amyloid may represent an abnormal accumulation of a normal, but ordinarily inconspicuous, tissue component, a possibility previously considered by Benditt and Eriksen.[16] The fact that areas of nonspecific birefringence do not fluoresce when stained with thioflavin-T does not rule out this possibility, since fluorescence microscopy may be less sensitive than polarization microscopy in detecting small deposits of amyloid.

Of interest was the observation that some hematoxylin and eosin-stained sections exhibited foci of green birefringence both in the presence and absence of amyloidosis. In contrast, Masson-stained sections did not, illustrating the fact that the occurrence of green birefringence depends not only on the presence of appropriately spaced and oriented fibrils, but also on the chemical configuration of the dyes used to stain them.

Polarization microscopy of Congo-red stained sections provides a convenient, reliable, and exquisitely sensitive method for detecting amyloid. However, because it does not distinguish between specific and nonspecific green birefringence, it requires confirmation, even in the case of formalin-fixed tissues, which are least likely to exhibit nonspecific green birefringence. As shown in the present study and by others,[7,17] thioflavin-T fluorescence is an even less specific sign of amyloidosis. However, it is more sensitive and can be identified more easily than other confirmatory staining reactions, so that fluorescence microscopy of thioflavin-T-stained sections is the method of choice for confirming the presence of amyloid in foci that exhibit green birefringence.

Summary

Evidence is presented to show that, under conditions of intense illumination and appropriate orientation of sections during polarization microscopy, many Congo red-stained tissues, especially when fixed in agents other than formalin, exhibit foci of green birefringence in the absence of amyloidosis. Such foci can be demonstrated in the healthy tissues of infants and young adults, both in man and the rat, so that they cannot be attributed to the microdeposits of amyloid that occur during senescence and in association with certain disease states. It is concluded that, while the demonstration of green birefringence in Congo red-stained sections of tissue is a convenient, reliable, and exquisitely sensitive method for detecting amyloid, it is not pathognomonic, and, hence, requires confirmation by other techniques, pre-

ferably by fluorescence microscopy of adjacent thioflavin-T stained sections, before it can be accepted as unequivocal evidence of amyloidosis.

References

1. Missmahl, H. P., and Hartig, M. Polarisations optische Untersuchungen an der Amyloidsubstanz. (Optical polarization studies of amyloid substance) *Virchow Arch Path Anat 324*:489–508, 1953.
2. Cohen, A. S., Calkins, E., and Levene, C. I. Studies on experimental amyloidosis. 1. Analysis of histology and staining reactions of casein-induced amyloidosis in the rabbit. *Amer J Path 35*:971–989, 1959.
3. Heller, H., Missmahl, H. P., Sohar, E., and Gafni, J. Amyloidosis: its differentiation into perireticulin and peri-collagen types. *J Path Bact 88*:15–34, 1964.
4. Cohen, A. S. Amyloidosis. *New Eng J Med 277*:522–530, (contd, 7 Sept) 1967.
5. Wolman, M., and Bubis, J. J. The cause of the green polarization color of amyloid stained with Congo red. *Histochemie 4*:351–356, 1965.
6. Puchtler, H., Sweat, F., and Levine, M. On the binding of Congo red by amyloid. *J Histochem Cytochem 10*:355–364, 1962.
7. Rogers, D. R. Screening for amyloid with the thioflavin-T fluorescent method. *Amer J Clin Path 44*:59–61, 1965.
8. Saeed, S. M., and Fine, G. Thioflavin-T for amyloid detection. *Amer J Clin Path 47*:588–593, 1967.
9. Lillie, R. D. *Histopathologic Technic and Practical Histochemistry* (ed. 3). McGraw-Hill, New York, 1965.
10. Pearse, A. G. E. *Histochemistry: Theoretical and Applied* (ed. 2). Little, Boston, 1960.
11. Vassar, P. S., and Culling, C. F. Fluorescent stains, with special reference to amyloid and connective tissues. *AMA Arch Path (Chicago) 68*:487–498, 1959.
12. Ravid, M., Gafni, J., Sohar, E., and Missmahl, H. P. Incidence and origin of nonsystemic microdeposits of amyloid. *J Clin Path 20*:15–20, 1967.
13. Schwartz, P. Senile cerebral, pancreatic insular and cardiac amyloidosis. *Trans NY Acad Sci 27*:393–413, 1965.
14. Haust, M. D. Fine fibrils of extracellular space (microfibrils). Their structure and role in connective tissue organization. *Amer J Path 47*:1113–1137, 1965.
15. Janigan, D. T. Experimental amyloidosis. Structural relationships of amyloid and reticulin in tissue sections and isolated preparations. *Amer J Path 49*: 657–678, 1966.
16. Benditt, E. P., and Eriksen, N. Amyloid. 3. A protein related to the subunit structure of human amyloid fibrils. *Proc Nat Acad Sci USA 55*:308–316, 1966.
17. McKinney, B., and Grubb, C. Non-specificity of thioflavine-T as an amyloid stain. *Nature (London) 205*:1023–1024, 1965.

[Illustrations follow]

Legends for Figures

Fig 1. Primary amyloidosis. Congo red-stained section of Zenker-acetic-fixed kidney showing foci of green birefringence in an arteriole. Plane of polarized light, as in all subsequent figures: vertical. × 37.

Fig 2. Section shown in Fig 1 rotated 90° clockwise in the horizontal plane of the microscope stage; plane of polarized light unchanged. Note shift in location of green birefringence. × 37.

Fig 3. Same section as in Fig 2 with polarizers uncrossed by a few degrees. Note change in color of birefringence from green to red. × 37.

Fig 4. Needle biopsy specimen of liver in patient with choledocholithiasis; section fixed in Carnoy's solution and stained with Congo red. Note foci of green birefringence in wall of central vein and in reticulin fibers outlining sinusoids. × 224.

Fig 5. Needle biopsy specimen of liver in patient with sclerosing cholangitis. Section fixed in Carnoy's solution and stained with Congo red shows foci of green birefringence in reticulin fibers outlining sinusoids. × 244.

Fig 6. Surgical biopsy section of liver in patient with carcinoma of common bile duct. Section fixed in formalin and stained with Congo red shows foci of green birefringence within the connective tissue of an enlarged fibrotic portal triad and within the wall of a portal vein radicle. × 93.

Fig 7. Section of skin obtained at necropsy from H.G., a 9-day-old infant who died of renal failure secondary to polycystic kidney disease. Section fixed in Carnoy's solution and stained with Congo red shows foci of green birefringence within connective tissue of dermis. × 93.

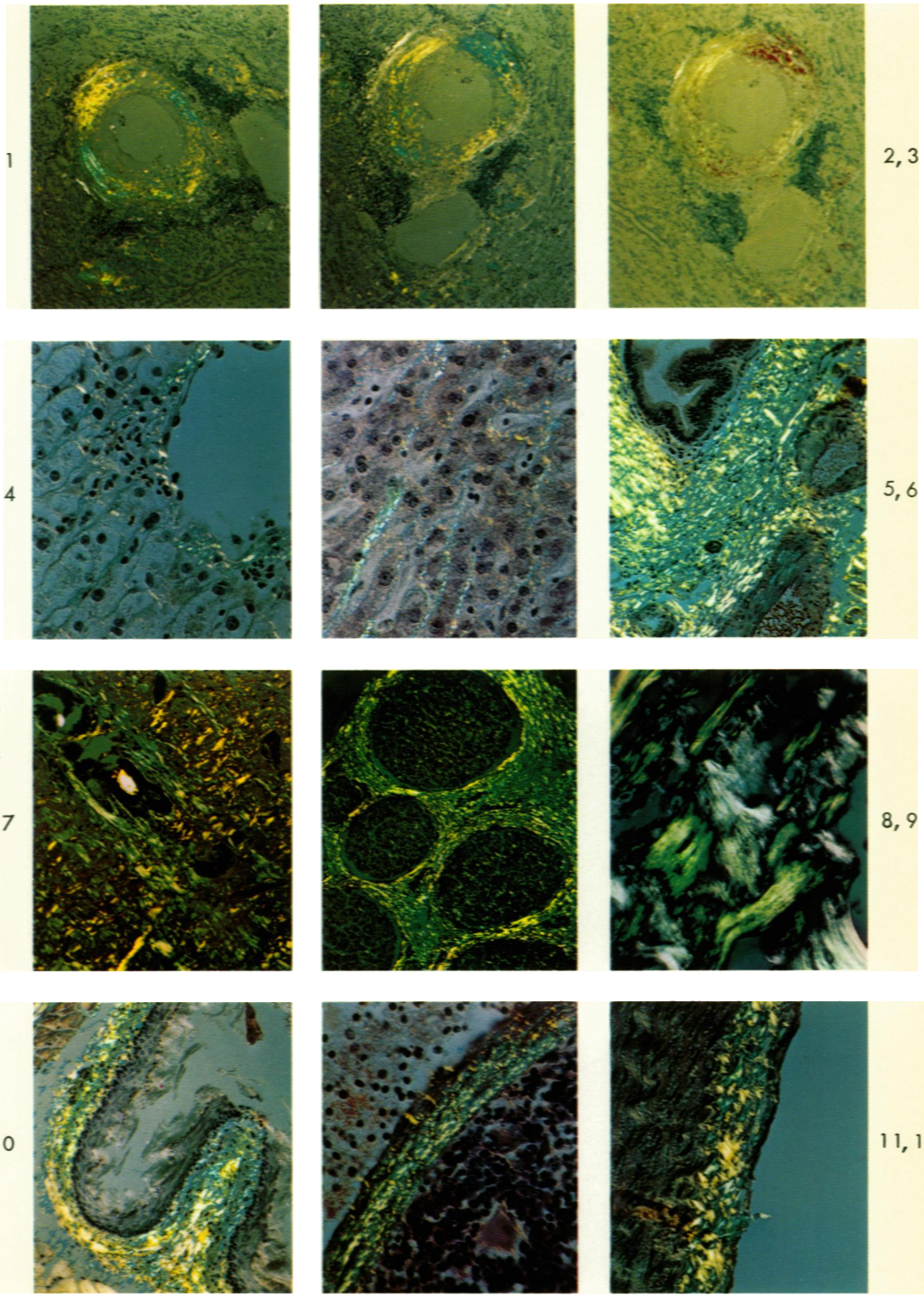
Fig 8. Specimen of nerve obtained at necropsy from H.G. Section fixed in Zenker-acetic solution and stained with Congo red shows foci of green birefringence in the perineurium. × 93.

Fig 9. Specimen of cancellous bone obtained at necropsy from A.T., a 9-day-old infant who died of congenital heart disease. Section fixed in Zenker-acetic solution and stained with Congo red shows numerous foci of green birefringence. × 244.

Fig 10. Specimen of esophagus obtained at necropsy from a healthy 400-g rat. Section fixed in absolute alcohol and stained with Congo red shows foci of green birefringence in submucosa. × 93.

Fig 11. Specimen of spleen obtained at necropsy from a healthy 60-g rat. Section fixed in Carnoy's solution and stained with Congo red shows foci of green birefringence in the wall of a large vein. × 244.

Fig 12. Specimen of aorta obtained at necropsy from a healthy 400-g rat. Section fixed in absolute alcohol and stained with Congo red shows foci of green birefringence in adventitia of aorta. × 93.


1
2, 3
4
5, 6
7
8, 9
10
11, 12